

Microbial Indicators of Nutrient Enrichment: A Mesocosm Study

R. Corstanje* and K. R. Reddy

ABSTRACT

Microbial communities are in close contact with the wetland soil microenvironment and can therefore function effectively as monitors of soil pollution. The objective of this study was to determine changes in the functional responses of microbial communities as a result of an external input of nutrients, while controlling for vegetation. A controlled experiment was performed at the mesocosm scale, consisting of two 1 m by 13 m raceways containing an organic peat soil, each planted with *Cladium* sp. and *Typha* sp. communities. One of the mesocosms was loaded with N ($2 \text{ g N m}^{-2} \text{ yr}^{-1}$) and P ($1 \text{ g P m}^{-2} \text{ yr}^{-1}$) for 18 mo. Nutrient loading resulted in increases in the soil and detritus labile nutrient pools, however, insufficient N and P were added to significantly alter their total levels. Over the experimental period, the extracellular enzyme acid phosphatase showed a significant decrease in activity across both plant communities ($P < 0.01$) in contrast to β -glucosidase activity, which varied primarily by plant community. Other microbial response variables such as the microbial activities (CO_2 and CH_4 production, $P = 0.0016$ and 0.0213 , respectively), microbial biomass ($P = 0.0018$) also varied primarily by vegetation type, with *Typha* sp. dominated areas exhibiting the highest level of activities. The nutrient dosing experiment indicated that the most immediate microbial response measures to nutrient enrichment are those directly associated to specific nutrients, such as P or N, while other measures showed a more complex response involving C source (e.g., vegetation type).

WETLANDS are important components in the landscape in that they are often the receiving bodies of point and nonpoint source contaminants. Major changes in wetland ecosystem structure can affect their capacity to function as contaminant sinks and transformers, rendering them less effective as sinks in the overall watershed. Nutrient enrichment has been shown to generate significant alterations to gross-scale wetland ecosystem structure and function (DeBusk et al., 1994; Davis et al., 2003) and induce changes in soil physicochemical and microbiological characteristics that then may serve as indicators of nutrient enrichment.

In natural systems, microbial communities have been shown to respond to N and P enrichment, such as increases in litter decomposition rates, observed in streams (Alexander, 1977; Qualls, 1984) and in wetlands (Davis, 1991; DeBusk and Reddy, 1998). Microbial responses to enhanced nutrient levels are also reflected in changes in indices of microbial activity, such as respiratory activities (Wright and Reddy, 2001) and extra-

cellular activities (Sinsabaugh and Moorhead, 1994). Eutrophication in marsh systems has also been associated with increases in microbially mediated N, C, and P turnover rates (Reddy et al., 1999), as well as increases in soil microbial biomass content (Qualls and Richardson, 1995). Experimental addition of N and P to natural systems (Elwood et al., 1981; Newbold et al., 1983) or to enrichment mesocosms (Qualls and Richardson, 2000; Newman et al., 2001) has produced significant microbial responses to the P and N additions, primarily as a function of their status as limiting factors in the systems under observation.

However, many of these studies were conducted on systems that had already undergone significant changes in plant community composition, affecting the litter quality and hence possibly the microbial community response. Litter quality has been significantly correlated with the microbial response measures. Factors such as the lignocellulose composition (DeBusk and Reddy, 1998) and the nutrient content of the plant litter material (Kögel-Knaber, 2002) all determine the response of the microbial communities in concert with a direct response to enhanced levels of nutrients.

Enclosed experimental systems (mesocosms) have been used extensively as a means to achieve controlled experiments at ecosystem level conditions (Kemp et al., 1980; Odum, 1984; Ives et al., 1996). Mesocosms have been used in aquatic environments to assess planktonic responses (Peterson et al., 1997, 1999) and they have also been used in wetlands to evaluate soil microbial community responses (Newman et al., 2001) as well as benthic responses to nutrient enrichment (McCormick et al., 1996). The objectives of this study was to experimentally determine the response of microbial communities to external N and P inputs in two different plant communities, *Typha* and *Cladium* sp.

MATERIALS AND METHODS

Mesocosm Design

Two experimental mesocosms were constructed in 1994 and filled with a locally obtained organic soil (histosol). The mesocosms were made of concrete and contained a double liner of heavy duty plastic. Each mesocosm was 13 m long by 1 m wide and filled to a 50-cm depth with organic soil obtained from Traxler Peat Co. (Palatka, FL). They were planted in 1994 with a gradient community ranging from a predominantly *Cladium jamaicense* plant community, grading into a *Typha latifolia* predominated area. At the beginning of the present experiment (date: 9/2000), additional wetland plant species present in the system were characterized as a mix of *Sagittaria lancifolia*, *Salix* sp., and *Scirpus* sp. in the intermediate zone between the *Typha* sp. and *Cladium* sp. areas. The plant com-

R. Corstanje and K.R. Reddy, Wetland Biogeochemistry Lab., Soil and Water Science Dep., Univ. of Florida-Institute of Food and Agricultural Sciences, 106 Newell Hall, P.O. Box 110510, Gainesville, FL 32611; R. Corstanje, current address: Rothamsted Research, Herts, AL5 2JQ, United Kingdom. Received 23 Feb. 2004. *Corresponding author (ron.corstanje@bbsrc.ac.uk).

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677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: DDI, distilled deionized; TC, total C; TN, total N; TP, total P.

munities were chosen to reflect a typical South Florida marsh system undergoing eutrophication, such as WCA-2a in the Everglades (Davis et al., 2003) or Blue Cypress Marsh at the headwaters of the St. Johns river (Corstanje and Reddy, 2004), in which the *Typha* sp. reflects enriched areas and the *Cladium* sp. the natural communities. After construction and establishment, the mesocosms were briefly used (in 1997) by students of a wetland biogeochemistry course, otherwise left untouched.

At the initiation of the experiment, these communities were well established and all mesocosms had achieved steady state. Each of the *Cladium* sp. and *Typha* sp. areas were divided into three 1.5-m² subsections, covering a total of 4.5 m² per species per mesocosm (Fig. 1). One of the mesocosms was randomly selected (consequently designated enriched), and pulse loaded with 2 g N m⁻² yr⁻¹ (NH₄Cl) and 1 g P m⁻² yr⁻¹ (KH₂PO₄) over the experimental period (18 mo), using a dilute solution (~0.4 μM P and ~2 μM N), pulse loaded once a week, for a total cumulative load of 13 g of P and 26 g of N. The enriched mesocosm was fitted with a sprinkler system placed across the center of the mesocosm with three heads placed at 1.5-m intervals along the mesocosm center axis over the sections planted with *Typha* sp. and *Cladium* sp., respectively (Fig. 1). A parallel study was initiated by placing litterbags containing *Typha* sp. and *Cladium* sp. litter in the respective plant community areas (Corstanje et al., 2006).

Both mesocosms were fitted with a recycling system that was initiated on loading to ensure complete mixing of the water column in both mesocosms, and to ensure similar experimental conditions over all mesocosms. Water was drawn from the water column in the center of the mesocosm at about the 20-cm depth and reintroduced at either end of the mesocosm with an average total water column volume recycle of

2 h (Fig. 1). Water levels were also maintained constant by introduction of tap water during the recycling period.

Soil Sampling and Analysis

Within each of the three 1.5-m² sections, detritus and soil were randomly sampled, resulting in three detritus and three soil cores per plant community per sampling event. The detritus sampled was the easily distinguishable plant material that was no longer attached to the parent plant. Detritus was sampled with a square frame ($a = 400 \text{ cm}^2$) and encompassed an average depth of the 5-cm depth. The underlying soil was sampled using a 10-cm diameter stainless steel corer; the edge of the corer was sharpened with undulating teeth to minimize compaction. This design allowed the corer to ride over roots as the corer was manually rotated, cutting versus snagging the root material. Experimentation with this and other corer designs indicated that this design resulted in least compaction (~15%) despite the small corer diameter. The soil cores were sectioned in 0- to 5- and 5- to 10-cm depth intervals and immediately brought to the laboratory and stored in the dark (4°C) until further analysis.

Sampling was executed every 3 mo, over a period of almost 2 yr, three sampling periods before loading and four sampling events in the ensuing 18 mo. Soil and detrital samples were homogenized in a grinder after removal of any visible live plant material such as roots. Soil bulk density was determined on a dry weight basis (70°C).

Total P (TP), C (TC), and N (TN) concentrations were determined on oven dried (70°C), ground samples; TC and TN with a Carlo-Erba NA 1500 CNS Analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Total P was determined by the

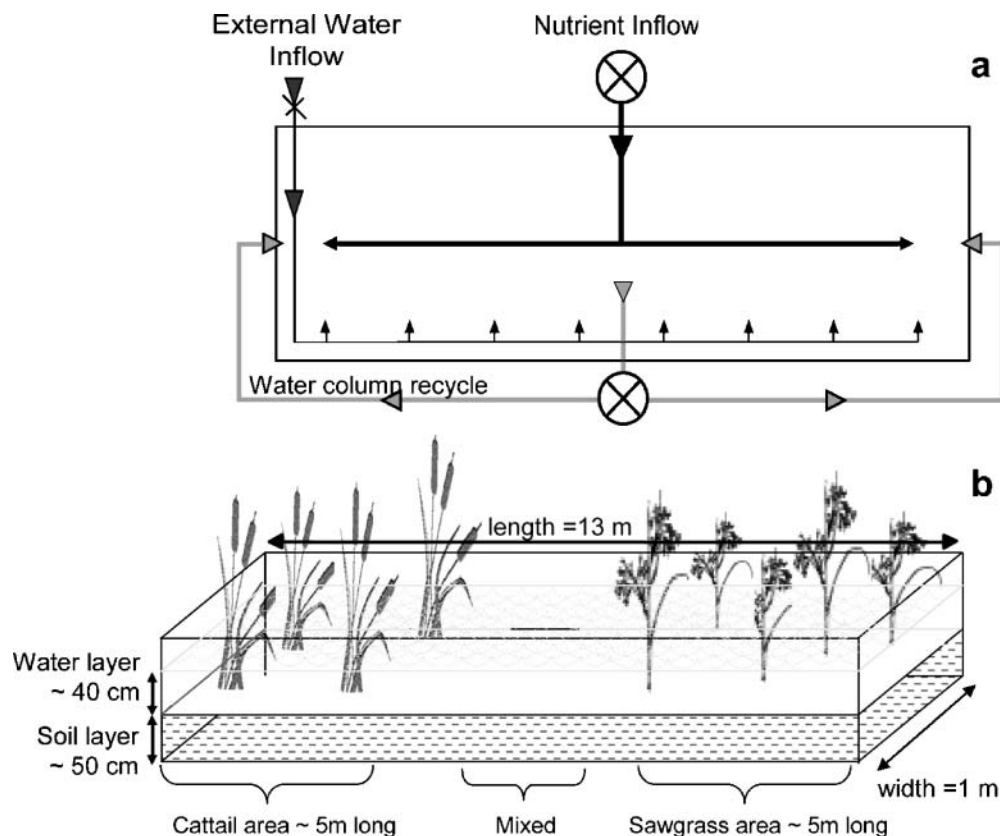


Fig. 1. Schematic overview of the enriched mesocosm, with the top view (a) illustrating the main water inputs and side view (b) representing the main dimensions.

TP ashing method and analyzed by the ascorbic acid colorimetric procedure (Kuo, 1996; Technicon Autoanalyzer II; Terrytown, NY).

Microbial activities were measured on soil slurries during anaerobic incubation, and were prepared by placing 5 g of sample in 27-mL anaerobic tubes (Bellco Glass, Vineland, NJ) with 10 mL of deionized distilled (DDI) water. The tubes were capped with butyl stoppers-aluminum crimps (Wheaton, Millville, NJ) and the soil slurry was actively purged with O₂-free N₂. They were subsequently placed horizontally in the dark at 28°C. Shaking was set at 180 rpm. Earlier work had demonstrated that this does not significantly affect methanogenesis (D'Angelo and Reddy, 1999). Samples were preincubated for 2 wk to ensure complete anaerobiosis. Upon completion, the headspace was purged again with O₂-free N₂. Initial conditions were established in terms of headspace pressure and CO₂ and CH₄ content. Subsequently the samples were incubated for 4 d under the previously described conditions and the CO₂ and CH₄ headspace content monitored. Headspace CO₂ was measured through thermal conductivity detector (TCD; Shimadzu 8AIT GC, Shimadzu Corp., Kyoto, Japan) and headspace CH₄ was analyzed by means of flame ionization detection (FID; Shimadzu 8AIF GC, Shimadzu Corp., Kyoto, Japan) as described in D'Angelo and Reddy (1999).

Microbial biomass C was determined by chloroform fumigation incubation procedure coupled to a 0.5 M K₂SO₄ extraction (Vance et al., 1987; White and Reddy, 2001). The extracted dissolved organic C was determined on a Shimadzu Total Organic Carbon analyzer (TOC-5050A). Microbial biomass C was calculated using the extraction efficiency factor $k_{EC} = 0.37$ (Sparling et al., 1990) as the difference between treated (fumigated) and untreated soils.

Microbial biomass P, potentially mineralizable P and N were only determined on the final ($t = 18$ mo) sample. Microbial biomass P was determined by fumigation extraction, using 25 mL of 0.5 M NaHCO₃ extractant. The difference in TP between the treated and untreated sample constitutes microbial biomass P, no extraction efficiency factor was used. The control was reported as 0.5 M NaHCO₃ extractable P, or labile organic P (Ivanoff et al., 1998). Potential mineralizable N was measured as a contrast of control and 10-d readings using 0.5 M K₂SO₄ extract (automated colorimetric analysis; EPA365.1, Technicon Autoanalyzer), the control was reported as K₂SO₄ extractable NH₄-N. Potential mineralizable P was determined by means of a 10-d anaerobic incubation. Equivalent of 0.5 g dry weight soil sample were placed in 50-mL serum bottles and mixed with 5 mL of DDI, capped and purged with O₂ free N₂. The samples were subsequently incubated in the dark at 40°C for 10 d. Upon termination of this period, 20 mL of 1.0 M HCl was injected in the serum bottle and after a 3-h extraction, filtered (0.45 μ m) and stored at 4°C until analysis. A second set of samples of equivalent weights (controls) was directly extracted with 25 mL (vs. 20 mL) of 1.0 M HCl as described previously. The HCl extract was analyzed on a Technicon Autoanalyzer (Terrytown, NY) by the ascorbic acid colorimetric procedure (Kuo, 1996). The difference in HCl-extractable P over the 10-d incubation period constitutes potential mineralizable P (mg P kg⁻¹ d⁻¹), the control was reported as total inorganic P (TPI).

The extracellular enzyme activities of β -1,4-glucosidase (EC 3.2.1.21) and acid phosphatase (EC 3.1.3.1) were assayed using a fluorescent artificial substrate methyl-umbelliferone (MUF-phosphate and MUF- β -D-glucoside respectively). Briefly, a 1 g to 20 mL soil slurry was made and further homogenized using a Tissue Tearor (Fisher Scientific, Pittsburgh, PA). Subsequently 200 μ L of a 1/100 dilution of this soil slurry was transferred to eight wells of a 96-well microtiter plate and 50 μ L of substrate added to four wells (and four blank). Plates

were incubated at room temperature (25 \pm 2°C) for 2 h for phosphatase, and for 24 h for β -glucosidase. Enzyme activity was expressed as the mean difference in fluorescence reading (Bio-Tek FL600 fluorometric plate reader, Bio-Tek Instruments, Inc., Winooski, VT) between the blank and sample over the incubation period (Prenger and Reddy, 2004).

Data Analysis

The CO₂ and CH₄ production were analyzed as zero-order kinetic reactions and estimated as the coefficient of simple linear regression (Micosoft Excel 2000, Microsoft Corp., Redmond, WA). Enzyme activities were normalized to a 0 to 1 range by dividing by the highest value obtained for that particular enzyme (E_p : normalized phosphatase; E_c : normalized β -glucosidase, Sinsabaugh et al., 1997) and then expressed proportional to the activity of β -glucosidase resulting in the factor E_p/E_c .

To be able to distinguish the effect of the treatment from the intrinsic variability present between and within the units, the units were monitored over three sampling events (9 mo) before loading one of the units, which, being analogous to 'plot' in the classical experimental design, is understood as pseudo-replication. Subsequently, the treatment effect and plant effect were nested within a time effect. For each soil layer, the models applied had general form:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + (\alpha\beta\gamma)_{ijk} + \epsilon_{ijkl} \quad [1]$$

where, y_{ijkl} = response measure for i -th treatment, j -th plant community and k -th time, l -th rep; μ = overall mean; α_i = effect due to the i -th treatment effect, and assumed to be normally distributed with mean zero and standard deviation $\sigma_{\alpha<\gamma}$; β_j = effect due to the j -th plant community, and assumed to be normally distributed with mean zero and standard deviation $\sigma_{\beta<\gamma}$; γ_k = effect due to the k -th month, and assumed to be normally distributed with mean zero and standard deviation σ_{γ} ; ϵ_{ijkl} = residual effect, assumed to be normally distributed with mean zero and standard deviation σ_e .

In this form, the model generated estimates for treatment (df = 1), plant (df = 1), plant \times treatment interaction (df = 1) and plant \times treatment within month (df = 18), and LSD for the pair wise comparisons. Residual Maximum Likelihood (REML) mixed effects models were used in all analyses (MIXED procedure in SAS, Version 9.0, 2003, SAS Institute, Inc., Cary, NC and GenStat, Version 8.1, 2005, Lawes Agricultural Trust). Fixed model terms were tested using the Wald test, which is asymptotically distributed as chi-squared with the degrees of freedom associated to each of the terms. As the mesocosms were repeatedly sampled, the data represent time series, an additional model that allowed the residual terms to be autocorrelated was examined. If significant autocorrelation exists and is not accounted for in the model, the variance components may underestimate the true variability and hence provide a false description of true (long-run) variability in the response. Autocorrelation was tested by comparing the deviance estimates (log-likelihood ratio statistic) of the models with and without an autoregressive term (AR1). If the differences between the deviances fell under a χ^2 threshold of 3.84, including the AR term was deemed unnecessary. The data were examined for normality and homoscedascity of variance and natural log transformed where necessary. The variance was reported as (\pm) standard deviations.

RESULTS

Soil pH did not vary considerably across mesocosms, depths or plant types, remaining on average close to pH

neutral (6.95). The bulk density of the soil in the enriched unit (0.24 g cm^{-3}) was somewhat higher than that in the control unit (0.20 g cm^{-3}), the detrital bulk densities were similar in both units (0.02 and 0.03 g cm^{-3} ; enriched vs. control, respectively). The physico-chemical characteristics of these soils are generally comparable to most other wetlands dominated by histosols (White and Reddy, 2001).

In comparing the TP levels in the two mesocosms before loading, *Typha* sp. detrital TP levels equaled $1220 (\pm 47)$ and $1007 (\pm 235) \text{ mg kg}^{-1}$ and *Cladium* sp. levels were $536 (\pm 88)$ and $510 (\pm 65) \text{ mg kg}^{-1}$. Similarly, the average soil TP levels were $493 (\pm 60)$, $597 (\pm 93)$, $477 (\pm 33)$, and $535 (\pm 61) \text{ mg kg}^{-1}$ for the *Cladium* sp. and *Typha* sp. areas of the control and enriched mesocosms, the two mesocosms did not differ by strata or vegetation over the period before loading. Nutrient loading did not affect the final concentrations of TP, TC, TN, or TC/TN in soil or detritus. They differed primarily between vegetation type (24:1, 33:1 for *Typha* sp. and *Cladium* sp., respectively) and over soil depth (24:1, 27:1 for detritus and soil, respectively). Similarly, the nutrient loading did not result in any changes in the C/N/P ratios. The C/N/P ratios of the detrital layer were 368:17:1 for *Typha* sp. and 663:19:1 for *Cladium* sp. Likewise the C/N/P ratios of the 0- to 5-cm soil layer were 661:28:1 for *Typha* sp. and 967:35:1 for *Cladium* sp. The average C/N ratio was 30:1 for *Cladium* sp. compared to an average of 29:1 for *Typha* sp.

Assuming that all the P and N was retained in the top 0- to 10-cm surface soil and detritus layer and that

Table 1. Total phosphorus and nitrogen by mass in the enriched and control mesocosms.

Mesocosm	Vegetation	Depth	TP		TN	
			— g —		— kg —	
			Mean	SD	Mean	SD
Organic Enriched	<i>Typha</i> sp	Detrital†	7.7	1.1	0.1	0.0
		0–5 cm	42.7	2.5	1.6	0.1
		5–10 cm	27.8	3.2	1.3	0.3
	<i>Cladium</i> sp	Detrital†	5.8	1.9	0.1	0.0
		0–5 cm	21.6	2.9	0.8	0.2
		5–10 cm	34.8	9.6	3.2	0.3
Organic Control	<i>Typha</i> sp	Detrital†	9.8	0.9	0.2	0.0
		0–5 cm	21.4	9.3	0.6	0.1
		5–10 cm	19.5	7.2	1.0	0.2
	<i>Cladium</i> sp	Detrital†	6.7	0.7	0.1	0.0
		0–5 cm	16.2	1.4	0.6	0.1
		5–10 cm	18.8	0.1	0.8	0.4

† Over an average of a 5-cm depth.

detritus had an average depth of approximately 5 cm, the overall P pools in the enriched mesocosm averaged $140 (\pm 21) \text{ g}$ for the enriched mesocosm, compared with an overall P pool of $92.4 (\pm 19.5) \text{ g}$ in the control mesocosm. A similar increase in the enriched mesocosm was noted for N (Table 1). This in contrast to the overall similarity of TP soil concentrations across the mesocosms, possibly alluding to the difference in the soil bulk density across the two units.

Increases in labile organic P were found for only the detrital layer of the enriched mesocosm, with higher levels in the *Typha* sp. areas (414 ± 82 and $260 \pm 43 \text{ mg kg}^{-1}$; enriched vs. control) when compared with the

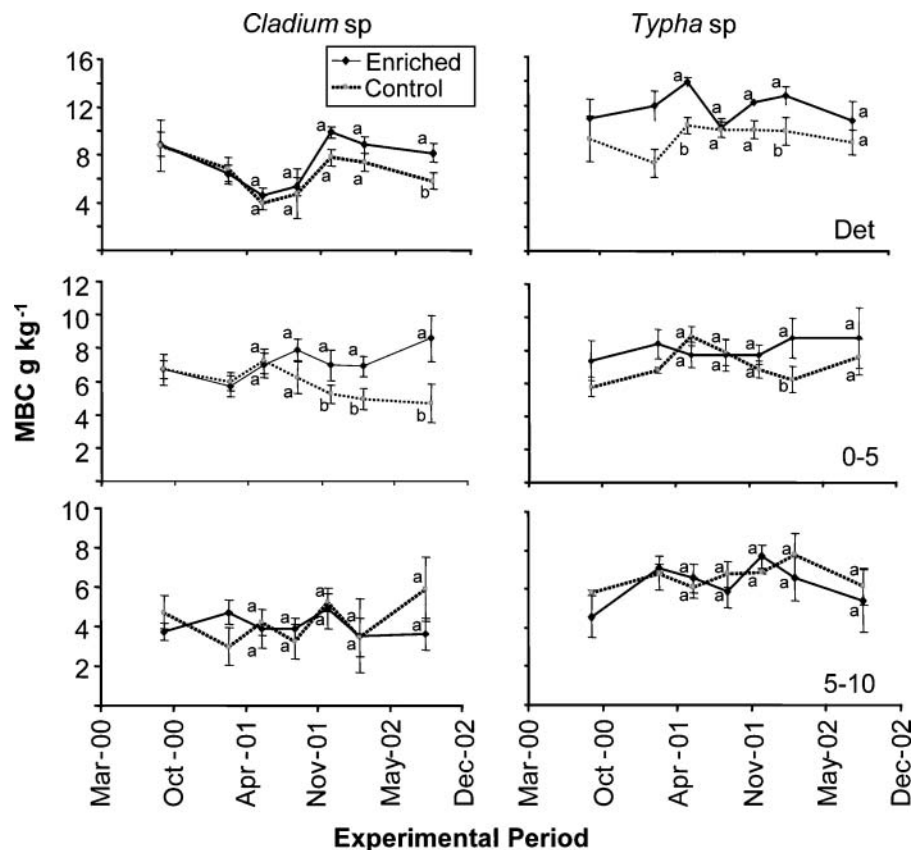


Fig. 2. Microbial biomass carbon (MBC) content in the two mesocosms, presented by depth in the two vegetation communities (*Cladium* sp. and *Typha* sp.) over seven different sampling events.

Cladium sp. (223 ± 41 and 122 ± 31 mg kg⁻¹; enriched vs. control). This effect was reflected in the increases in TP_i levels in the detrital layer over the two vegetation types (70 ± 2.2 and 55.5 ± 4.9 mg kg⁻¹; enriched vs. control, respectively), changing little in the deeper soils. The levels of K₂SO₄ extractable NH₄-N varied across all depths and vegetation types, with an average level of NH₄-N in the enriched mesocosm 60 mg kg⁻¹ (± 6.5) and in the control 39 mg kg⁻¹ (± 6.7).

The nutrient loading resulted in the increases in the levels of potentially mineralizable forms of N and P. The detrital material in the *Typha* sp. areas contained most of the mineralizable P (17 ± 1.8 and 14 ± 8.9 mg kg⁻¹ d⁻¹; enriched vs. control respectively) and N (19 ± 4.8 and 14 ± 1.6 mg kg⁻¹ d⁻¹; enriched vs. control, respectively) compared with PMP (17 ± 1.8 and 14 ± 8.9 mg kg⁻¹ d⁻¹; enriched vs. control, respectively) and PMN (12 ± 0.6 and 10 ± 4.1 ; enriched vs. control) in the *Cladium* sp. areas. The average soil PMP and PMN were also found to be higher. The levels of microbial biomass, both in terms of their P and C content, were consistently higher in the *Typha* sp. detritus and shallow soils (0–5 cm) across both units. This difference was not affected by the nutrient loading. Upon termination of the experiment, there were higher levels of microbial biomass P in the enriched unit

(90.33 ± 21.67 mg kg⁻¹) when compared with the control (45.67 ± 4.2 mg kg⁻¹). On the other hand, levels of microbial biomass C at the termination of the experiment were not affected by the nutrient loading (Fig. 2) when compared to the initial values (Sept-00 and March-01) with the exception of the microbial biomass C levels in the surface (0–5 cm) and detrital layers in *Cladium* sp. toward the end of the experimental period. The subsequent statistical analysis indicated a highly significant plant effect on microbial biomass C throughout all layers ($P < 0.001$), whereas the treatment effect alone was highly significant ($P < 0.001$) in the detrital layer and the plant \times treatment \times month interaction was highly significant ($P < 0.001$) in the soil layers. All other terms were not found to be significant.

The associated C turnover rates (CO₂ production and CH₄ production rates; Fig. 3 and 4) exhibited very similar responses to plant type over the different depth intervals. The microbial activities were consistently higher in the detrital layer and decreased with depth. In the case of CO₂ production the detrital layer showed a significant treatment response ($P = 0.080$), while in the case of CH₄ production, the plant \times treatment interaction term was significant ($P < 0.001$). This is reflected in that for the CO₂ production in detritus, the final

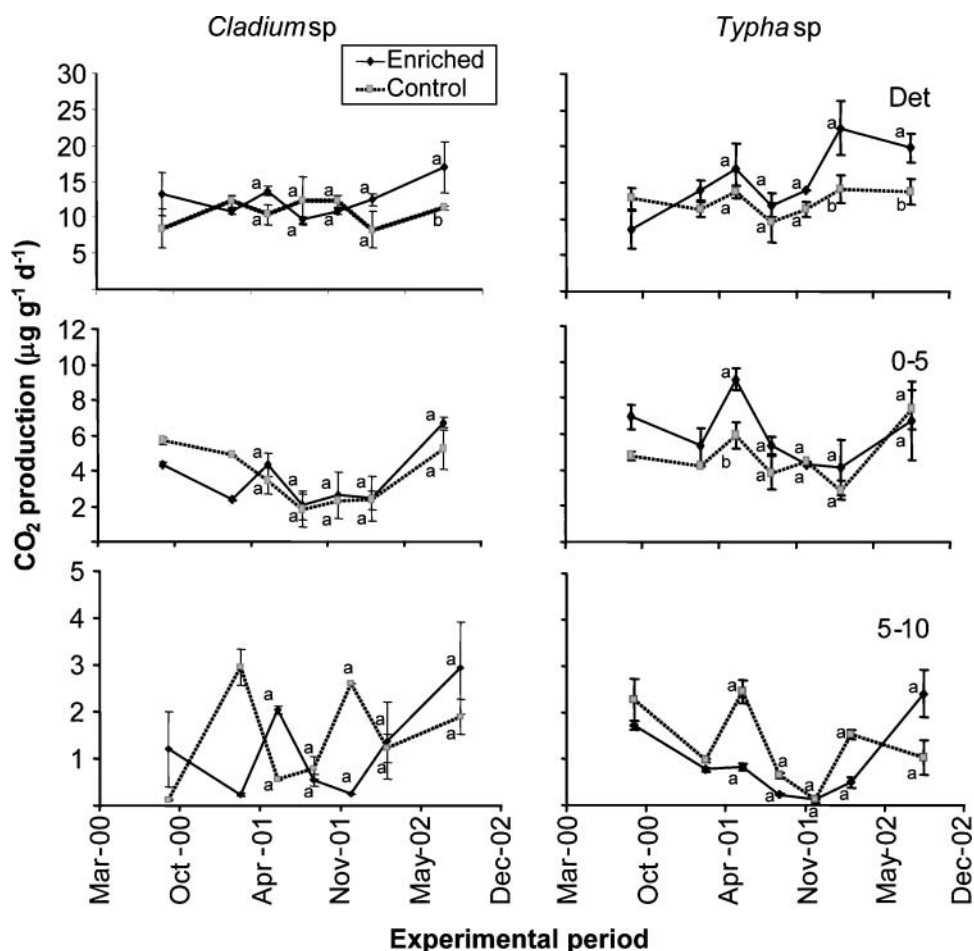


Fig. 3. Microbial activities (CO₂) in the two mesocosms, presented by depth in the two vegetation communities (*Cladium* sp. and *Typha* sp.) over seven different sampling events.

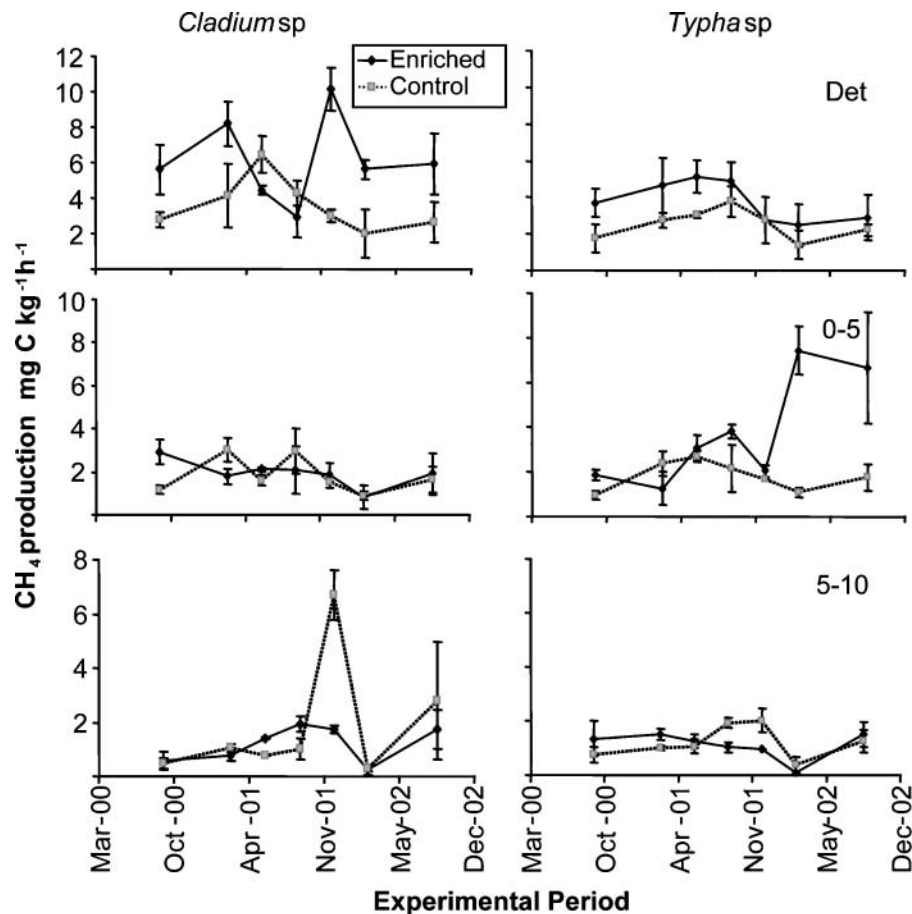


Fig. 4. Methane production (CH_4) in the two mesocosms, presented by depth in the two vegetation communities (*Cladium* sp. and *Typha* sp.) over seven different sampling events.

measurements are significantly higher for samples from the enriched unit, while for the CH_4 production rates this is only the case for *Cladium* sp. In the soil layers, the top 0- to 5-cm layer a significant treatment \times plant \times time interaction for CO_2 ($P < 0.001$). In the case of methanogenesis, a significant treatment \times plant interaction term for CH_4 ($P = 0.006$) was found, a result of a reverse the trends shown in the detrital layers, where *Typha* sp. soil produced more CH_4 than *Cladium* sp. soils. The microbial activities in the deeper soils (5–10 cm) showed little response to either plant type or nutrient enrichment, with no significant model terms.

The extracellular enzyme acid phosphatase (Fig. 5), in contrast to β -glucosidase activity (Fig. 6), shows a substantial decrease in activity as a function of the nutrient loading over detrital and top 0- to 5-cm surface soil. At all layers, the treatment term was significant and plant \times treatment \times month interaction terms was found to be significant ($P < 0.001$ in all cases). These results are driven by the decrease in acid phosphatase activity that was found in the enriched mesocosm and the increased phosphatase activities that were found associated with *Typha* sp. when compared with *Cladium* sp. Analysis of the β -glucosidase activity reflected a general lack of response of the enzyme to the nutrient additions. A significant plant type term ($P < 0.001$) was found in the detrital layer, β -glucosidase activities where on average higher in *Typha*

sp. detrital material than in *Cladium* sp. The soil layers showed no response to either plant type or nutrient treatment. Both the acid phosphatase as the β -glucosidase enzyme activities decreased with depth.

DISCUSSION

Although the advantage of using these units was that the plant communities and soils were well established, there were only two units, preventing true replication. Parts of this study, therefore, fall within the realm of pseudoreplication, in which experimental units are treated as independent when they are not (Hurlbert, 1984). This would be the case if we were to use statistical models on the analyses (potential mineralizable P, extractable P, etc.) executed on samples obtained for the final sampling event. The standard deviations presented for these properties represent the within plant within unit variability, the residual errors of the observations are not independent. However, while this experimental design contained non-independent observations, for those properties measured before and after the treatment we were able to describe their respective treatment effects appropriately.

Nutrient enrichment has been noted to generally increase organic matter mineralization rates (Davis, 1991; Qualls and Richardson, 2000) including the mineraliza-

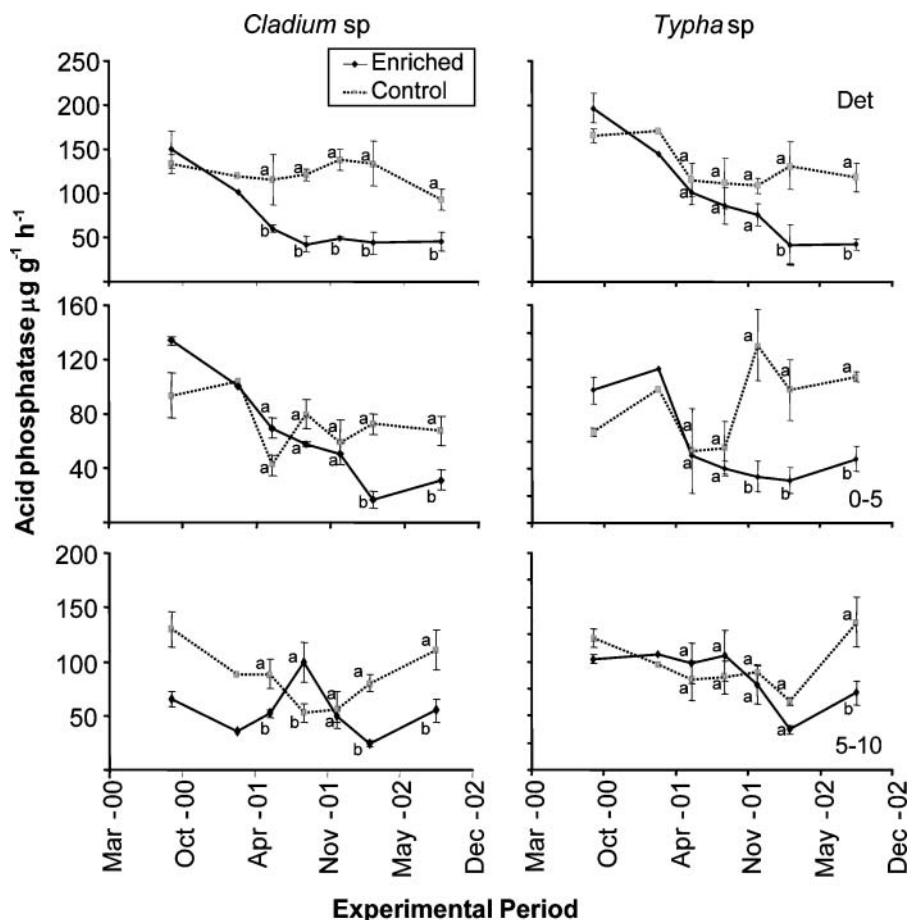


Fig. 5. Acid phosphatase activities in the two mesocosms, presented by depth in the two vegetation communities (*Cladium* sp. and *Typha* sp.) over seven different sampling events.

tion of organic P (Bridgham et al., 1998). These increases in mineralization rates could result in increased levels of available nutrients, which in peat soils can further fuel the eutrophication process. The differences across vegetation classes by mesocosms reflect plant tissue N/P ratios as found throughout a range of environmental conditions (5:1 to 15:1 for *Typha* sp. and >50:1 for *Cladium* sp.; Boyd and Hess, 1970; Koch and Reddy, 1992). In all mesocosms there we observed a net potential for N mineralization (Williams and Sparling, 1988), that is, N was probably not limiting in these systems. Nutrient loading resulted in an increase in the mineralizable P fraction in the surface layer (Fig. 7, Panel C), the mineralizable N and biomass P and C fractions did not increase as a result of the nutrient loading. The average overall turnover of P in the enriched mesocosm is 118 (± 117) d, that in the control mesocosm 320 (± 427) d. The associated N turnover rates are 1630 (± 680) d and 2964 (± 2186) for the enriched and control mesocosm, respectively.

The proportion of basal respiration (CO_2 production) to microbial biomass C, that is, the metabolic coefficient $q\text{CO}_2$ (Anderson and Domsch, 1990), has been identified as a sensitive response variable to soil organic matter quality (Kaiser and Heinemeyer, 1993; Meyer et al., 1996). The suggestion is that a large $q\text{CO}_2$ coefficients are an indication of disturbed ecosystems (Dilly et al.

1997), in which microbial communities respire more per unit biomass than in stable systems. Similarly, potential mineralizable P (PMP) is the amount of P released into solution after a short (10-d) anaerobic incubation. It is assumed that this is primarily microbially mediated (Bridgham et al., 1998) and reflects potential P-turnover rates on site. It is also a function of the biodegradability of the organic P. The microbially mediated turnover rates of P and C in these mesocosms indicate a general increase in the P turnover rate as a result of the P loading while there appeared no effect of the nutrient loading on the metabolic coefficient (Fig. 7). The nutrient loading as such did not seem to generate stress as to affect the microbial C metabolism, but did increase P metabolism.

The synthesis of enzymes is regulated by the presence or absence of the readily available substrates. Presumably, the production of enzymes is relatively expensive at a cellular level, resulting in a hydrolytic activity that reflects the relative need of the microbial communities present in each mesocosm (Sinsabaugh et al., 1997). If production of extracellular enzymes is cast in terms of resource allocation by the microbial communities, the relative activity of N and P-acquiring enzymes contrasted to the permanent C requirement is an indication of the levels of N or P limitation that these microbial communities experience in that particular environment (MARCIE model, Sinsabaugh and Moorhead, 1994).

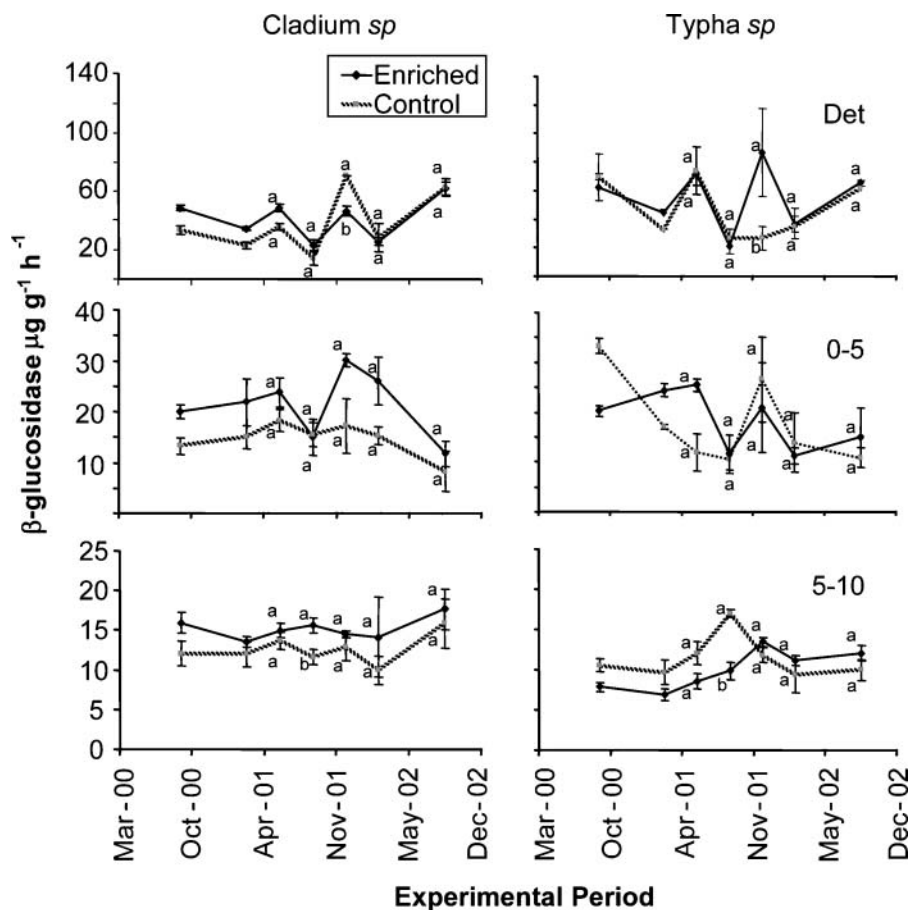


Fig. 6. β -glucosidase activities in the two mesocosms, presented by depth in the two vegetation communities (*Cladium* sp. and *Typha* sp.) over seven different sampling events.

As a result of the ongoing nutrient loading, the ratio of acid phosphatase activity to β -glucosidase activity steadily decreases throughout the soil column in the enriched mesocosm (Fig. 8) compared with the control mesocosm, indicating shifting microbial requirements away from P acquisition. As an initial response to the

ongoing nutrient enrichment, the relative activity of extracellular enzymes responded quickly (3–6 mo) to the nutrient influx, particularly in the detrital layer.

Across all mesocosms, the areas dominated by *Typha* sp. consistently had higher overall microbial biomass and nutrient turnover rates. Similarly, the detrital component

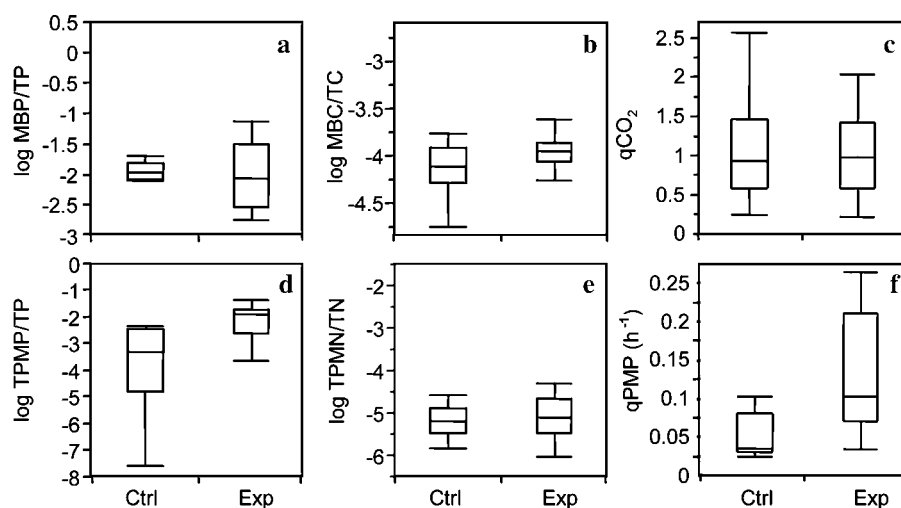


Fig. 7. Relative proportion of microbial biomass in the overall P, N pools (a,b), the metabolic quotient (c) (qCO_2 , Anderson and Domsch, 1990), the proportion of P and N turnover (PMP & PMN) as fraction of the total P and N pools (c,d) and the P turnover (PMP) per mesocosm biomass (e) in the surface soils (detritus to 5 cm's depth, Exp; Experimental; Ctrl; Control).

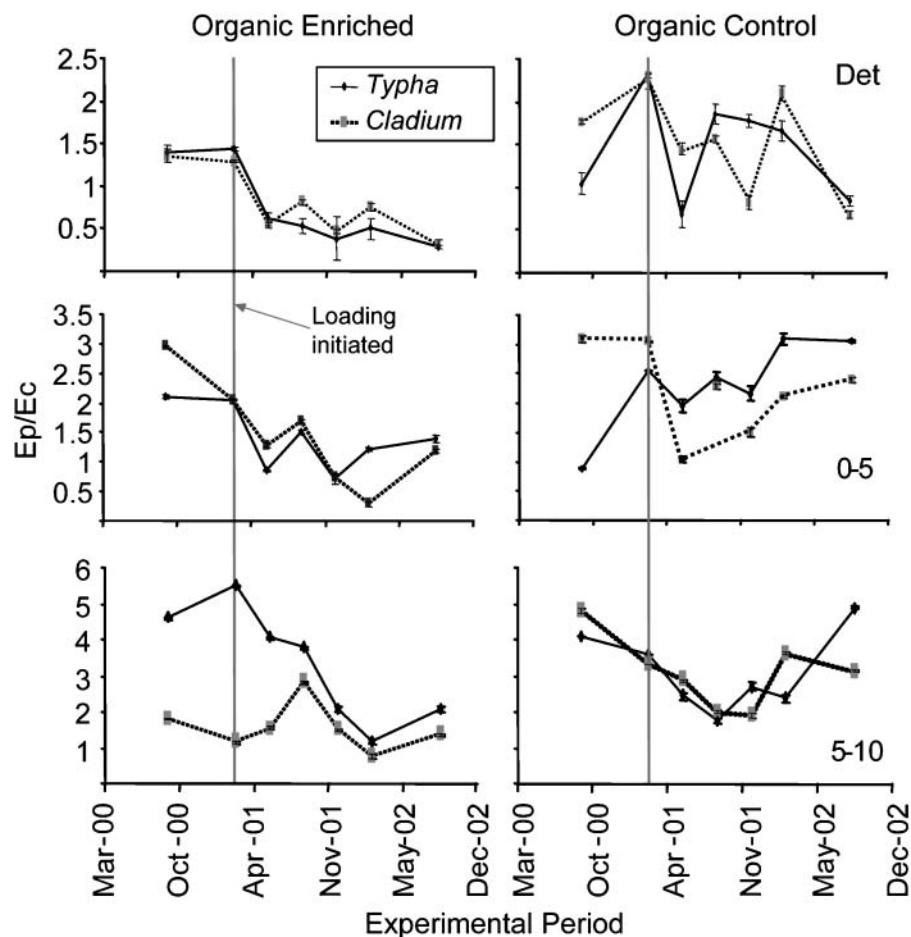


Fig. 8. Distribution of the relative proportion of acid phosphatase (E_p) to β -glucosidase (E_c) in the two mesocosms, by vegetation type.

was found to be far more dynamic and responsive than the bulk soil, which is consistent with other studies (Reddy et al., 1999). The litterbag study (Corstanje et al., 2006), executed in tandem with this experiment, illustrated the importance of litter source in decay rates and associated microbial community metabolic responses, with *Typha* sp. litter decay significantly faster than *Cladium* sp. litter. The litterbag experiments did show an enrichment effect on *Typha* sp. litter decay, no effect was noted on *Cladium* sp. litter decay.

All measures associated with C turnover, such as the MBC, CO_2 , and CH_4 production rates, primarily responded to vegetation type and depth versus the direct nutrient influx. In natural marsh systems, nutrient enrichment has been shown to increase aerobic (DeBusk and Reddy, 1998) and anaerobic (Wright and Reddy, 2001) C turnover rates; and increased loss of litter C (Qualls and Richardson, 2000) as a function of both litter type (*Typha* vs. *Cladium*) and water column P. We did not find any response to the nutrient enrichment in any of the C-related parameters. Amongst other factors, the decomposition of organic matter is governed by the chemical composition of the decomposing plant material (Kögel-Knaber, 2002), which have been shown to change under different nutrient conditions (Güsewell and Koerselman, 2002). Thus its effects on the soil biogeochemistry of nutrient impacted areas have been far

more significant than could be attained in this relatively short experimental timeframe. This divergence is significant, because it alludes to two types of biogeochemical response measures, a primary set of response measures to the perturbation (nutrient enrichment) and a secondary set of measures that are a function of the larger scale changes that result from nutrient enrichment (e.g., shifts in the dominant plant communities).

In conclusion, the measures that are most closely associated with the P cycle (alkaline phosphatase activity, potential mineralizable P, and microbial biomass P) were those that responded to the nutrient enrichment and responded within a relatively short time frame (<6 mo). The plant community type had a significant effect on all measures, with the *Typha* sp. detritus exhibiting the highest levels of potentially labile components and microbial community biomass, and activity. The external nutrient inputs had no effect on microbial biomass C, CO_2 , and CH_4 production rates or potential mineralizable N during the experimental period.

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